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INDUCTION OF *ASCOSPHAERA* (ASCOMYCETES: ASCOSPHAERALES) INFECTIONS IN FIELD POPULATIONS OF *OSMIA LIGNARIA PROPINQUA* CRESSON (HYMENOPTERA: MEGACHILIDAE)

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Abstract.—Offspring of field populations of *Osmia lignaria propinqua* Cresson were contaminated by forcing females to emerge through larval cadavers filled with chalk brood spores. Infection rates of subsequent larvae varied from 0 to 54% suggesting a varietal or biotype difference in the pathogenicity of *Ascosphaera torchioi* Youssef & McManus on *O. l. propinqua.* Differences were found in infection rates for bees from Reno, Nevada, and between bees from Reno and Logan, Utah. Chalk brood infection rates did not vary significantly by season or by cell construction sequence of nests.

Key Words.—Insecta, chalk brood, mycosis, disease, bees

Studies on the biology and pathogenicity of *Ascosphaera* spp. infecting the blue orchard bee, *Osmia lignaria propinqua* Cresson, have shown that six species of *Ascosphaera* will produce mycosis (Youssef et al. 1985). Developmental temperatures of the host affected rates of infection, with low temperatures increasing the mycosis. The number of spores ingested by feeding larvae also increased the prevalence of chalk brood (Rust & Torchio in press).

Ascosphaera spores, the infective agent of chalk brood syndrome, develop within densely packed spore cysts immediately below or on the surface of larval species cadavers (Stephen et al. 1981, Vandenberg & Stephen 1982, McManus & Youssef 1984, Youssef et al. 1985). Ingested spores germinate within the foregut of host bee larva and resultant hyphae penetrate the gut wall before the host larva dies (Vandenberg & Stephen 1983, McManus & Youssef 1984). As adult bees emerge from natal nests containing sibs killed by *Ascosphaera,* spores are dispersed on individuals as they move past or chew through these larval cadavers (Stephen & Undurraga 1978, Vandenberg et al. 1980, Stephen et al. 1981, Vandenberg & Stephen 1983).

Emerging alfalfa leafcutting bees, *Megachile rotundata* (Fabr.), can each carry more than 100 million *Ascosphaera aggregata* Skou spores, with the spore load depending on the number of larval cadavers contacted during emergence (Vandenberg et al. 1980). Stephen et al. (1981) found that spore loads carried by individual *M. rotundata* declined from several million at emergence to several thousand after three weeks of activities under field conditions. They also showed that spore counts in nesting domiciles increased as the season progressed.

Our field study was designed to determine infection rates of *O. l. propinqua* offspring whose mothers were forced to emerge from nests containing known

numbers of larval cadavers infected with *Ascosphaera torchioi* Youssef & McManus (Youssef & McManus in press), using larval cadavers obtained from Logan, Utah and Reno, Nevada. Rust & Torchio (in press) obtained spore loads of 1.0 to 6.0 billion from *O. l. propinqua* larval cadavers infected with *A. torchioi.* We thus hypothesize that the first emerging female to contact a larval cadaver will be more heavily contaminated than subsequent individuals crawling through the same cell during emergence. As a result, the first female to emerge from a nest contaminated with *Ascosphaera* spores should infect more offspring than subsequent females emerging from the same nest. Chalk brood infections also should be greater in larvae produced early in the nesting season by contaminated females than in larvae produced late in the season. In addition, larvae produced by uncontaminated females that reutilize contaminated nests should also have higher prevalence of *Ascosphaera* infection than larvae produced by uncontaminated females using new, clean nests.

Materials and Methods

1988 Study.—Nest traps of *O. l. propinqua* from Reno were x-rayed to determine nest-cell occupancy. Nests containing *Ascosphaera* infected larval cadavers were carefully dissected and the cell contents were removed. Cell partitions and nest plugs were left undisturbed. Thirteen larval cadavers were weighed to 0.1 mg and placed in the outer-most cells of 10 infected nests (seven with one cadaver each and three with two cadavers each. Twenty-five adult *O. l. propinqua* females were weighed, individually color coded, returned to their cocoons, and placed in inner cells of the 10 infected nests (one in one nest, two in three nests, and three in six nests). Ten additional cadaver-free nests were assembled with each containing five male *O. l. propinqua* as mates for the emerging females. A second population of 25 color coded females and 50 males assembled into 10 male and 10 female cadaver-free nests, was also prepared as controls.

The two populations were placed in the field on 1 May 1988 approximately 500 m apart and adjacent to Sagehen Creek, 15 km N of Truckee, California. Both were provided with clean nest traps (19 mm × 19 mm × 150 mm pine blocks drilled with 5–9 mm diameter holes to a depth of 130 mm). Nests were examined every other day for nesting, and active nests were coded for identification of marked females and dates of activity. Completed nests were returned to the laboratory on 2 Jul 1988. Nests were x-rayed and dissected during Nov to determine offspring production and survival or mortality. Ambient temperature ranges were recorded at the site.

1889 Study.—Nest traps of *O. l. propinqua* from Logan and Reno were prepared in a similar manner as described above; 26 contaminated nests were assembled, each with one *Ascosphaera* larval cadaver in the outer cell: Thirteen of these nests contained *Ascosphaera* cadavers recorded from Logan and the remaining 13 cadavers originated from Reno. Two female *O. l. propinqua* from Logan were placed in the inner cells of each nest. Twenty additional nests were prepared each with five males bees from Logan. The 13 *Ascosphaera* nests from Logan and 10 male nests were placed in a mixed fruit orchard in Logan on 8 Apr 1989 along with clean nest materials as described above. The 13 *Ascosphaera* nests from Reno and 10 male nests were placed in a mixed fruit orchard in Reno on 28 Feb 1989 along with clean nest materials. Nests were examined daily at both sites for nesting

Table 1. Experimental design, nesting results, and infection rates for *Ascosphaera torchioi* Youssef & McManus on field populations of *Osmia lignaria propinqua* Cresson.

Nest treatment		Number of nests	Bee source	Cadaver source	Experimental location	Number of nests	Number of cells	Number of larvae infected	Percentage
Bees	Cadavers								
				1988 Study					
3	1	3	Reno	Reno	Truckee				
3	2	3	Reno	Reno	Truckee				
2	1	2	Reno	Reno	Truckee	25	80	7	8.75
2	2	1	Reno	Reno	Truckee				
1	1	1	Reno	Reno	Truckee				
Control[a] 2	none	5	Reno	—	Truckee	4	10	2	20.00
Control 3	none	5	Reno	—	Truckee	35	114	0	0.00
				1989 Reno Study					
2	1	13	Logan	Reno	Reno	22	132	72	54.50
2	1	13	Reno	Reno	Reno	—[b]	—[b]	—[b]	—[b]
Control[c]			Reno	—	Reno	16	84	18	21.40
				1989 Logan Study					
2	1	13	Logan	Logan	Logan	16	103	15	14.50
2	1	13	Reno	Logan	Logan	—[b]	—[b]	—[b]	—[b]
Control[c]			Logan	—	Logan	18	117	11	9.40

[a]—Female nesting at treatment site.
[b]—No nesting, females died over winter.
[c]—Natural.

activity and active nests were coded for identification of marked females and dates of activity. Nests produced by unmarked females were used as field controls at both sites. Temperature and humidity were recorded at both sites. Nests were returned to the laboratory in Jul, x-rayed and dissected in Nov to determine offspring production and mortality.

The reciprocal test using Reno *O. l. propinqua* was prepared and the nests and bees were placed in the field. The Reno bees suffered severe overwintering mortality and only a few individuals emerged. One female began nesting but produced no offspring (RWR, unpublished data).

Chi-square test for goodness of fit and Student's *t*-test for comparing two population means were used. *Osmia l. propinqua* chalk brood cadavers representative of those used in this study are deposited at the USDA ARS Bee Biology and Systematics Laboratory, Logan, Utah.

Results

1988 Study.—Table 1 shows the experimental results. Ten of the 25 spore-contaminated *O. l. propinqua* females nested at the contaminated site (none nested at the control site). One control female nested at the *Ascosphaera* site, and these 11 females together produced 29 nests and 90 cells; averaging 3.1 ± 2.8 (range, 1–7) cells per nest. These females were exposed to 0–7.4 billion spores, and nine cells (10.0%) were infected with *Ascosphaera.* Mean *Ascosphaera* cells per nest was 0.3 ± 0.6 (0–2). Seventy-one cells in the remaining 22 nests had no chalk brood deaths.

Thirteen of the control females nested at the control site where 35 nests and

114 cells were produced; averaging 3.2 ± 2.1 (1–7) cells per nest. None of those 114 cells contained *Ascosphaera,* and no *Ascosphaera* infected larvae have been recovered from *O. l. propinqua* reared from the Sagehen Creek area during the three previous years of nest trapping (RWR, unpublished data). No differences were found in the mean cells per nest between contaminated and clean bees (t = 0.23, df = 62, P = 0.82).

Five contaminated females each produced one or more cells with chalk brood offspring, but the remaining five contaminated females produced chalk brood-free offspring. The one control female nesting at the contaminated site produced 10 cells in four nests of which two larvae were infected with chalk brood.

There was no difference in the occurrence of chalk brood in cells produced during first, middle, or last parts of the nesting season from that expected based on the number of cells completed during those intervals (χ^2 = 2.403, df = 2, 0.50 > P > 0.25). Also, there was no difference in the position of chalk brood cells within a nest when compared to the number of first, second, etc. constructed cells in a nest (χ^2 = 3.887, df = 5, 0.75 > P > 0.50).

1989 Logan Study.—Logan bees released through Logan *Ascosphaera* cadavers produced 16 nests with 103 cells, averaging 6.4 ± 1.5 (2–9) cells per nest. Fifteen (14.5%) chalk brood cadavers were recovered, or 0.9 ± 0.9 (0–3) cadavers per nest.

Eighteen nests and 117 cells were produced by clean Logan bees averaging 6.5 ± 1.9 (2–9). Eleven (9.4%) offspring were infected with *Ascosphaera* or 0.6 ± 1.1 (0–4) cells per nest. No clean or contaminated bees nested in the contaminated nests.

The Logan bees released in Logan showed no differences between mean number of *Ascosphaera* deaths or average number of cells in any of the treatments (*Ascosphaera* deaths—first to second emerging bees: t = 1.69, df = 14, P = 0.11; *Ascosphaera* to clean bees: t = 0.86, df = 32, P = 0.40; cells per nest—*Ascosphaera* to clean bees: t = 0.10, df = 32, P = 0.92; first to second emerging bees: t = 2.0, df = 14, P = 0.066). In addition, no differences were found in frequency of *Ascosphaera* infection during the first, middle, or later periods of the nesting season from that expected based on the number of cells constructed during the same intervals (*Ascosphaera* bees: χ^2 = 0.045, df = 2, P > 0.95; *Ascosphaera* bees emerging first: χ^2 = 0.061, df = 2, P > 0.95; *Ascosphaera* bees emerging second: χ^2 = 0.49, df = 2, 0.90 > P > 0.75; clean bees: χ^2 = 2.45, df = 2, 0.50 > P > 0.25). There was, however, one significant difference in the position of *Ascosphaera* cells within a nest compared to the number of first, second, etc. constructed cells in a nest: contaminated bees that emerged first produced more first (1, 2) and last (6, 7) constructed cells having *Ascosphaera* infected offspring than expected (χ^2 = 14.0, df = 6, 0.05 > P > 0.025).

1989 Reno Study.—Twenty-two nests with 132 cells were produced in Reno by Logan bees emerging through Reno *Ascosphaera* cadavers. Of these, 72 *Ascosphaera* cadavers (54.5%) were recovered with 3.4 ± 2.2 (1–8) cells per nest. Bees emerging first produced 11 nests and 63 cells with 47.6% (30) *Ascosphaera* mortality with 2.7 ± 2.1 (1–5) *Ascosphaera* cells per nest. Bees emerging second produced 10 nests and 69 cells from which 42 *Ascosphaera* larval cadavers were recovered (60.8%) with 4.2 ± 2.1 (1–8) chalk brood deaths per nest. There was, however, no difference in the mean number of *Ascosphaera* cells per nest produced by bees emerging first or second (t = 1.59, df = 19, P = 0.13).

Clean bees at Reno produced 16 nests and 84 cells with 5.2 ± 2.2 (2–8) cells per nest. Of these 18 *Ascosphaera* larval cadavers were found (21.4%) with an average of 1.1 ± 1.0 (0–3) per nest. Six nests with 24 cells were free of chalk brood.

There were no differences in frequency between contaminated and clean bees in the seasonal distribution of Reno *Ascosphaera* mortality, cell production, or cell distribution of *Ascosphaera* (seasonal distribution—*Ascosphaera* bees: $\chi^2 = 3.00$, df = 4, $0.75 > P > 0.50$; *Ascosphaera* bees emerging first: $\chi^2 = 6.33$, df = 4, $0.25 > P > 0.10$; *Ascosphaera* bees emerging second: $\chi^2 = 3.38$, df = 4, $0.50 > P > 0.25$; clean bees: $\chi^2 = 2.77$, df = 4, $0.75 > P > 0.50$; cell sequence—*Ascosphaera* bees: $\chi^2 = 1.78$, df = 7, $P > 0.95$; *Ascosphaera* bees emerging first: $\chi^2 = 1.86$, df = 7, $P > 0.95$; *Ascosphaera* bees emerging second: $\chi^2 = 2.13$, df = 7, $P > 0.95$; clean bees: $\chi^2 = 3.62$, df = 7, $0.90 > P > 0.75$).

Ascosphaera mortality increased whenever nesting populations were exposed to the Reno chalk brood spores. These differences were highly significant with Reno *Ascosphaera* killing more than three times as many offspring ($t = 4.19$, df = 35, $P = 0.0002$). There was a highly significant difference in the average *Ascosphaera* cells between the Reno *Ascosphaera* bees and clean bees ($t = 3.83$, df = 35, $P = 0.0005$). There was no difference in the cells constructed per nest between the two populations ($t = 1.45$, df = 35, $P = 0.15$). There was no difference in the average number of cells constructed per nest between Logan and Reno bee populations ($t = 0.25$, df = 35, $P = 0.80$).

Logan bees began nesting on 15 Apr and continued to nest for 27 days. During this period, the average hours of adult activity, based on temperatures ≥15.7° C (Torchio 1985) was 3.7 ± 2.6 (0–7) h per day. Bees in Reno began nesting on 22 Mar and continued for 30 days with an average of 5.0 ± 4.0 (0–10) working h per day ≥15.7° C. The hours available to adults for nesting were not different between the sites ($t = 1.35$, df = 55, $P = 0.18$). The average hours per day ≥21° C for the first 60 days of larval development (Rust & Torchio in press) was not different between Logan and Reno (Logan 2.6 ± 3.3 (0–10); Reno 2.6 ± 3.1 (0–9); $t = 0.14$, df = 118, $P = 0.89$). Rust and Torchio (in press) found that larvae reared at 21° C required more than twice the time to complete their development to the fifth instar when compared to larvae reared at 29° C (5–6 versus 12–14 days). Also, the highest *Ascosphaera* infection rate was obtained when larvae were reared at 21° C. Larvae reared below 21° C have a decreased survival rate.

Discussion

Three unexpected sets of results were obtained from these experiments. First, neither clean or contaminated females utilized nests from which they emerged. We could not, therefore, test the effect of these nests as a source of *Ascosphaera* contamination. However, contaminated nests were visited by known clean females (1988 Study) that then nested in clean holes in which 20% *Ascosphaera* mortality was measured (two of 10 cells) that accounted for 22% of the total *Ascosphaera* mortality at the site. If the wild, native bees were in fact free of *Ascosphaera* spores before searching for suitable nest holes, then some of these bees became contaminated while exploring the cavities from which the experimentally contaminated bees had emerged.

Second, our prediction that females emerging first through a larval cadaver

should infect more of their offspring but at a decreasing rate was not generally supported by the data except for one nest cell sequence (1989 Logan). Although there were more first constructed cells with chalk brood in the Logan nests, there were almost equal numbers of chalk brood cadavers in last constructed cells and fewer than expected cadavers in middle cells. Thus, there does not seem to be a seasonal decline in infectivity potential even though spore load has been shown to decline with time in the alfalfa leafcutting bee (Stephen et al. 1980). Spore loads obtained by emerging bees can be sufficiently high to contaminate cells at the same frequency throughout the nesting periods.

Third, there was an unexpected significant difference in the infection rates between Logan and Reno *A. torchioi* spores. Reno *A. torchioi* spores infected almost four times (3.7) as many offspring as did Logan *A. torchioi* spores. Yet, spore loads, numbers of spores per cadaver, nesting periods, temperatures during nesting and larval development, average number of cells constructed per nest, and number of nesting females were similar for both bee populations. These results suggest that there may be a difference in the infectivity potential of spores from each location. Perhaps Reno spores germinate more readily, or hyphae penetrate the gut wall more rapidly, or rapid mycelial growth assures increased host mortality. Future studies on comparative pathogenicity are required to determine potential varietal differences in *A. torchioi* in nests of *O. l. propinqua.*

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